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PURIFICATION AND CHARACTERIZATION OF BROMOCRESOL PURPLE FOR SPECTROPHOTOMETRIC SEAWATER ALKALINITY TITRATIONS

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PURIFICATION AND CHARACTERIZATION OF BROMOCRESOL PURPLE FOR SPECTROPHOTOMETRIC SEAWATER ALKALINITY TITRATIONS

By

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ABSTRACT

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Purification and Characterization of Bromocresol Purple for Spectrophotometric Seawater Alkalinity Titrations

Faculty Mentor: Dr. Michael DeGrandpre

The topic of my research is the purification of the sulfonephthalein indicator bromocresol purple (BCP). BCP is used as an acid-base indicator in seawater alkalinity determinations. Impurities in sulfonephthalein indicator salts often result in significant errors in pH values[3]. High-precision inorganic carbon (i.e. CO_2) measurements are necessary to document the changes in ocean chemistry and pH due to anthropogenic CO_2 uptake by the earth's oceans[3]. In order to reduce the error in seawater pH determinations and the general understanding of the CO_2 cycle, BCP must be purified and characterized. Previous work has described the use of flash chromatography to efficiently produce large batches of purified indicator, so this method was used for BCP purification[3]. Since BCP is purified at a level useful for seawater pH calculations, the chemical can be characterized by more accurately calculating the constants associated with it[1]. The spectrophotometric pH values obtained by measuring absorbance ratios are directly related to indicator molecular properties, such as molar absorptivity ratios and protonation characteristics[2]. Once the accuracy of these values is improved, future and archived spectrophotometric pH data can be calculated using the improved indicator equilibrium and molar absorptivity values[2]. The available purification method of BCP will advance the understanding of ocean acidification in systems where the chemical is used.

Purification and Characterization of Bromocresol Purple for Spectrophotometric Seawater Alkalinity Titrations

Introduction

Background

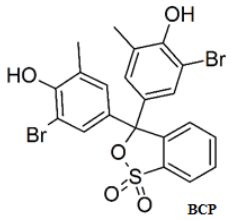


Figure 1: Bromocresol Purple (BCP) structure

Anthropogenic climate change is a relevant and crucial issue being addressed by the global population and scientific community. The broad topic encompasses numerous variables that form the overall change in global and regional climate patterns. One specific concern is ocean acidification (OA). OA is the ongoing decrease in the pH of the earth's oceans due to the uptake of anthropogenic carbon dioxide (CO_2) from the earth's atmosphere through the carbon cycle. Acidity or pH is inversely related to alkalinity. Technological advances have been made that allow scientists to monitor this issue via data collection in the atmosphere and in the ocean itself. Earth-orbiting satellites, such as NASA's Orbiting Carbon Observatory-2 (OCO-2) measures atmospheric CO_2 and transmits the data to scientists for analysis, thus improving the overall understanding of the carbon cycle and its impact on OA. A significant amount of the CO_2 released by humans into the atmosphere is absorbed by the oceans[4]. When gaseous CO_2 is dissolved in the ocean, it reacts with water (H_2O) to form carbonic acid (H_2CO_3) and various other species which consequently acidifies the oceans. The overall reaction can be represented by Equation 1:

$$H_2O\,(l) + CO_2(aq) \overset{Ka}{\leftrightarrow} H_2CO_3(aq)$$

One method of monitoring ocean pH or alkalinity is through use of an in situ titration apparatus that is placed directly in the ocean and transfers data to researchers for analysis. A Submersible Autonomous Moored Instrument (SAMI ^{TM}) manufactured by Sunburst Sensors in Missoula, MT measures oceanic pH or alkalinity, and provides valuable time-series data to researchers[4]. In order for this instrument to function, a sulfonephthalein indicator must be used to quantify pH during the titration and gain the necessary data for ocean acidification calculations[4]. One indicator that is used in the SAMI's ^{TM} is bromocresol purple (BCP) (Figure 1) which comes as a reagent in the form of BCP sodium salt (Figure 2) at roughly 90% purity, depending on the manufacturer[4]. This level of indicator impurities can introduce systematic

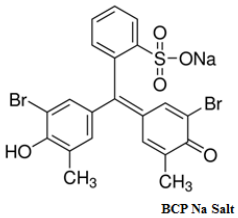


Figure 2: Bromocresol Purple (BCP) sodium salt structure

errors in reported spectrophotometric pH measurements, even when the precision level in the measurements is quite good[2]. Furthermore, archived spectrophotometric pH data has the potential to be quantitatively revised when the indicator is characterized[2]. To characterize the indicator means the pK_a, equilibrium constants, and molar absorptivity ratios are known with more accuracy[2]. Systematic differences in reported pH values were as large as 0.01 pH units, which on a logarithmic scale and with respect to the sensitivity of ocean chemistry to pH indicates a large error in reported pH values[2]. No BCP characterization or purification methods have been published, which led to my project of formulating a procedure for purification, and the ultimate characterization of the compound. To fully realize the advantages of spectrophotometric pH measurements and their applicability to the overall understanding of climate change, the issue of indicator impurities and their impact on accuracy and precision of measurements must be carefully studied and approached.

Problem

Bromocresol purple sodium salt (BCP) is a readily available sulfonephthalein indicator with roughly 90% purity directly from Sigma-Aldrich. The level of contaminants present in BCP and the relatively small amount used in spectrophotometric titration measurements makes purified BCP an ideal component of a seawater titration system of interest. The level of impurities present in the stock BCP potentially adds a large margin of error to calculations, specifically at the absorbing wavelength of 434 nm, which needs to be minimized. I developed a procedure for BCP purification that is concise and reproducible for researchers using the chemical. In addition, a quality control method has been made to verify the effectiveness of the purification. Previous work has described the use of flash chromatography (FC) to efficiently produce large batches of purified indicator, so this method was used to form a procedure for BCP purification[3]. By using an FC system, an effective procedure for purifying stock BCP has been generated, which is verified through HPLC-UV/Vis data analysis. Now that BCP has been purified at a level useful for seawater pH calculations, the indicator can be characterized by more accurately calculating the molar absorbance ratios and equilibrium constant values. This work will be completed in the near future.

Experimental Methods

Reagent and Solvents

The bromocresol purple (BCP) indicator, as a 90% pure sodium salt, was obtained from Sigma-Aldrich (batch MKBQ3276V). HPLC Grade Acetonitrile (ACN), HPLC Grade Methanol (MeOH), and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich and used without further purification. Nanopure water was used for all dilutions. Since indicator impurities can vary between batches even from the same manufacturer[2], and since plenty was available, one batch of indicator was used to allow for comparison between impure and pure indicator. Two solvents were compared to see which has the potential to more effectively separate the contaminants, and ultimately ACN was chosen for the final purification procedure. The use of TFA acidifies the indicator solution that is placed on the column, guaranteeing that the BCP is completely in the first protonated form, with the absorbing wavelength of 434 nm.

Instrumentation

Figure 3: Flash Chromatography System CombiFlash ® Rf 200

The BCP purification trials used in this study were performed on a Flash Chromatography System CombiFlash ® Rf 200 (FC), using both 5.5 and 275 g Redisep Rf C18 Aq columns from Teledyne-ISCO. The 5.5 g column was used for gradient optimization trials during the formation of the procedure, and the 275 g column was used for larger purification batches, and final verification of the procedure. Quality control was performed on a High Performance Liquid Chromatography instrument with a UV-Vis detector (HPLC-UV/Vis), Agilent 1100 Series, using an Agilent Eclipse Plus 3.5 µm 4.6 x 100 mm C18 Aq column. The columns on both instruments use a nonpolar C18 stationary phase, with an acidified polar solvent (ACN with 0.10% TFA) being run through as a mobile phase. The decision to use a nonpolar column and a polar solvent causes portions of the mixture which are more polar and have an affinity for the polar solvent to elute first, and then the most nonpolar to elute last.

Figure 4:HPLC-UV/Vis Agilent 1100 Series HPLC

In addition, BCP is soluble in the organic polar solvent ACN.

Trials

Using the theoretical basis for purification of a related sulfonephthalein indicator, metacresol purple (MCP), available in the literature[2], as well as an available procedure in the lab for purification, I began a series of 23 trials to optimize a solvent gradient for BCP. The trials addressed which acidification level worked best, 0.05% or 0.10% TFA in solution for the indicator and solvents, whether MeOH or ACN worked better, and if the smaller column being used for trials to conserve indicator and solvent was representative of the larger column. Results showed that 0.10% TFA acidification level of ACN had better results, and that the smaller 5.5 g was representative of the 275 g column, and reasonable to use for gradient optimization trials. In addition, the solvent profile or gradient was altered accordingly to resolve a contaminant peak and have it be fully separated from the purified BCP indicator peak. It was difficult to separate the purified indicator from the contaminants, and some overlap of the peaks remains.

Procedure: Purification Method

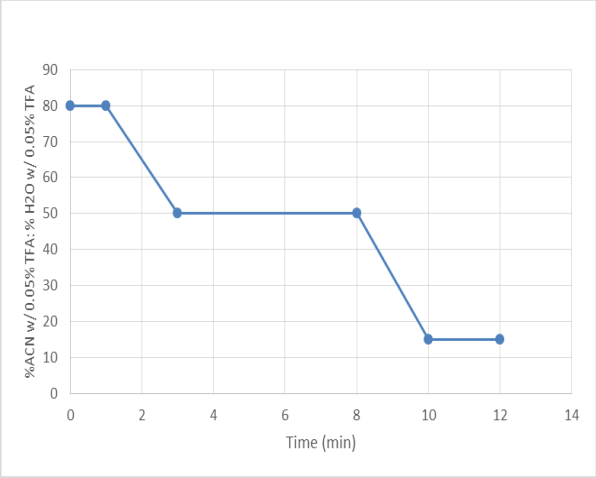


Figure 5: FC conditioning gradient BCP

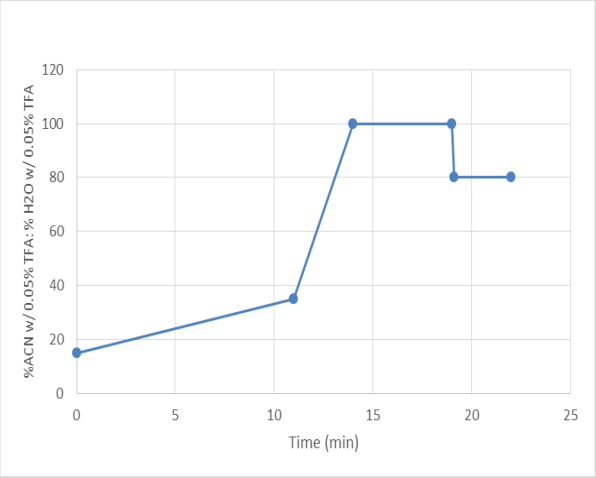


Figure 6: FC purification gradient BCP

Based on information from each trial, and an in-depth analysis of collections from each portion of the peaks, a final procedure was created. The purification procedure begins with guaranteeing the solvent bottles are full with A: H_2O:0.10 % TFA and B: ACN:0.10% TFA. The A and B positions correspond to the varying degrees of solvent seen in Figures 5 and 6. Prior to indicator solution injection on the column, the column must be conditioned to clear the column of previous contaminants and put the column at starting conditions for the procedure. The column used for large purification batches is the 275 g AqRedisep Gold C18 Aqua column, which is placed on the FC system. Figure 3 shows the conditioning procedure; the column is

stored in 80% ACN:20% H_2O without TFA, so the conditioning begins at 80% ACN, and the starting conditions in Figure 6 correspond to the end conditions in Figure 5. This consistency prevents shocking the column and prevents indicator from bleeding down the column.

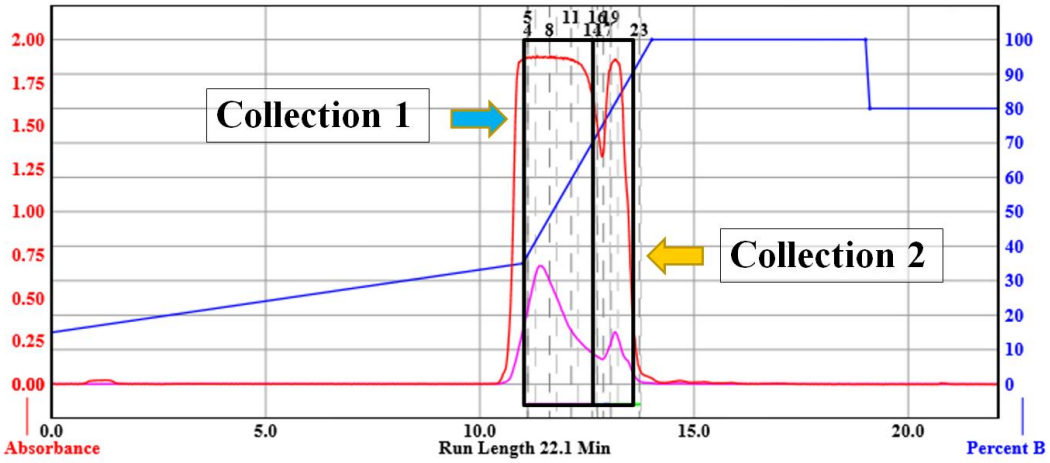


Figure 7: Chromatogram for representative purification on FC system

The BCP sample is prepared by dissolving 0.5-1.3 g in 100 mL of 85% H_2O:15% ACN:0.10% TFA in a 100 mL volumetric flask. This solution mimics starting conditions in the method seen in Figure 6. The range of mass for the sample was determined based on the loading capacity of the column (275 mg to 5.5 g), and what the initial trials indicated to work best for proper resolution of the purified indicator peak, Collection 1 in Figure 7, from the contaminant peak, Collection 2 in Figure 7. Absorbance, which is measured on the y-axis in Figure 7, is a function of concentration based on the Beer-Lambert Law, Equation 2: $A=\varepsilon bc$, where A=absorbance, ε=molar absorptivity constant or extinction coefficient, b=path length of the cell, and c=concentration. This theoretical basis serves as an indication that as concentration increases, absorbance increases proportionally. Although the column can reasonably have 5.5 g loaded, the increased concentration causes more peak overlap than seen in Figure 7, which reduces the effectiveness of the procedure and the purification level of the product.

Column	**C18Aq 275 g RediSep Gold**
Flow Rate	150 mL/min
Wavelengths of Detection	210, 254 nm
Mass of Impure BCP	1.1217 g
Solution	0.10% TFA:15% ACN:85% H2O

Table 1: Purification conditions on FC for trial shown in Figure 7

In the representative example of the purification procedure being discussed in this paper, the conditions for the run are shown in Table 1. The column, flow rate, wavelength of detection, and solution are consistent with all purifications, the mass of the sample is specific to the chromatogram in Figure 7. After the column is conditioned and the indicator solution is made, the solution may be loaded onto the column manually or via syringe. In my research, I have manually loaded the column. Once the column is prepared, the method is loaded on the FC system which uses the gradient shown in Figure 6. In Figure 7, Collection 1 is the purified indicator, and Collection 2 is mostly contaminants. This is verified on the HPLC-UV/Vis.

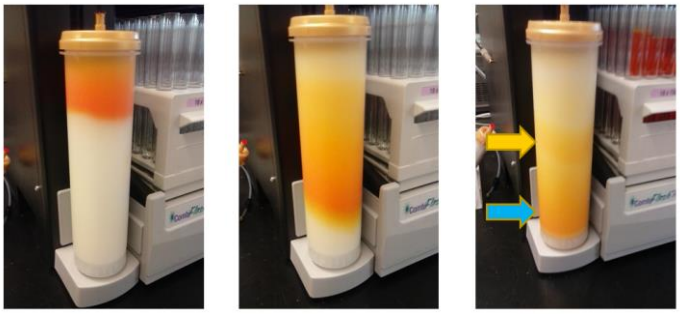


Figure 8: Indicator and impurity bands on 275 g AqRedisep Gold

Figure 8 shows the column at various times during the purification shows in Figure 7. At 12.3 minutes the arrow on bottom corresponds to Collection 1 in Figure 7, and the arrow on top corresponds to Collection 2. Once the run is complete, and the column is clear of any contaminants, the column is stored in 80% ACN:20% H_2O without acid to prevent the column from degrading.

The purified indicator at this point is in solution, and the solvent needs to be evaporated off to have a solid product. The drying may be done via a rotary evaporation system or the samples may be placed in a ported Erlenmeyer flask, and dried with stir at 40^{o}C with filtered air blown over the solution. Once the solvent is evaporated off, the solid product is transferred to its final container, and dried in an oven at 50^{o}C for 3 to 6 hours. The product is light sensitive so it is stored in an amber UV protective vial in a desiccator.

Procedure: Quality Control

Once the product is dried, it must be verified that it has been purified and no unusual contaminants have been added throughout the drying process. A small sample, around 10 mg, is placed in a 10 mL volumetric flask and dissolved in a 5% ACN:95% H_2O:0.05% TFA solution to mimic starting conditions on the column. The exact mass is not analytically relevant, since quantification and percent yield is not performed on the HPLC-UV/Vis quality control (QC) data. The method used for QC on the HPLC-UV/Vis is shown in Figure 9. This method is similar to the one used on the FC, but not the same. This method has been optimized for use on the

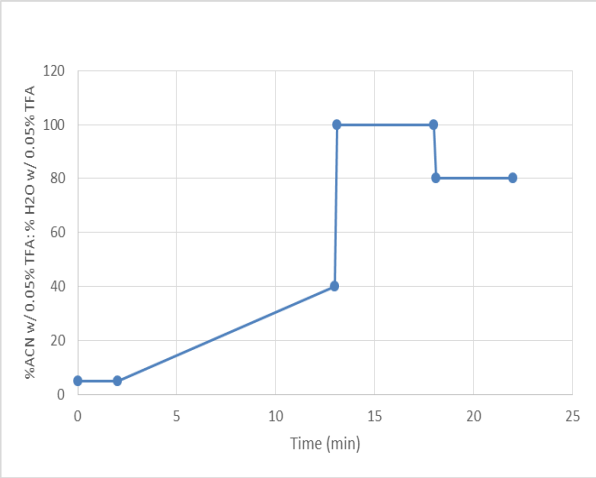


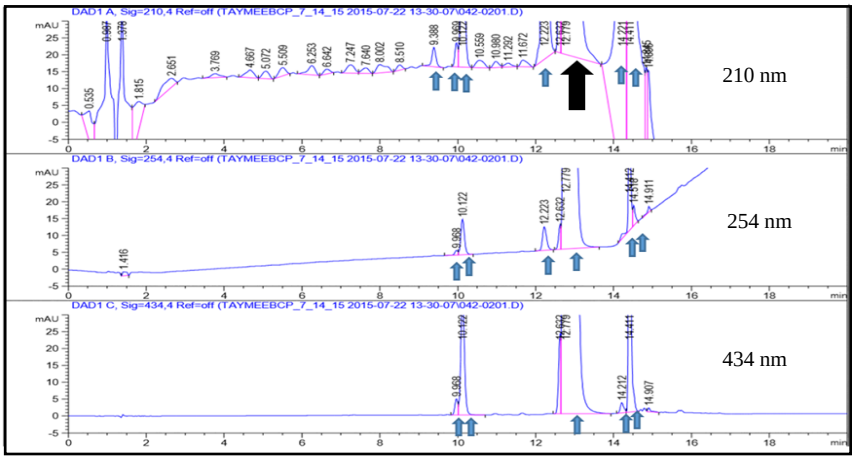
Figure 9: HPLC-UV/Vis QC gradient

system, and uses a much smaller sample of 10 µL. The small sample, combined with the length of the column, allows for clear and separate peaks on the chromatograms the system generates. The UV/Vis detector allows for the analysis of each individual peak. With the spectra of each individual peak available, the indicator peaks can be distinguished from the contaminants. This is shown in Figure 10, which is the HPLC-UV/Vis analysis of the Collections 1 and 2 shown in Figure 7, compared to an impure BCP sample.

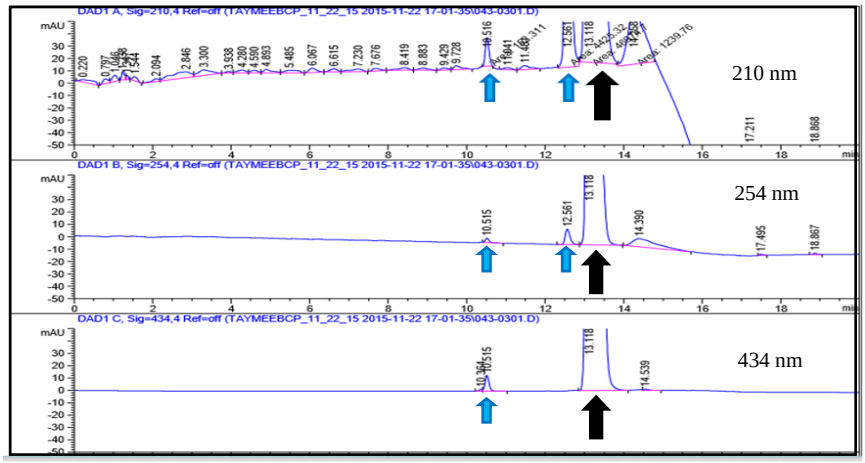
Column	**Eclipse Plus C18 4.6 x 100 mm**
Flow Rate	1 mL/min
Wavelengths of Detection	210, 254, 434 nm
Volume of Injection	10 µL
Solvents	A: H_2O w/ 0.05% TFA B: ACN w/ 0.05% TFA

Table 2: Quality control conditions on HPLC-UV/Vis

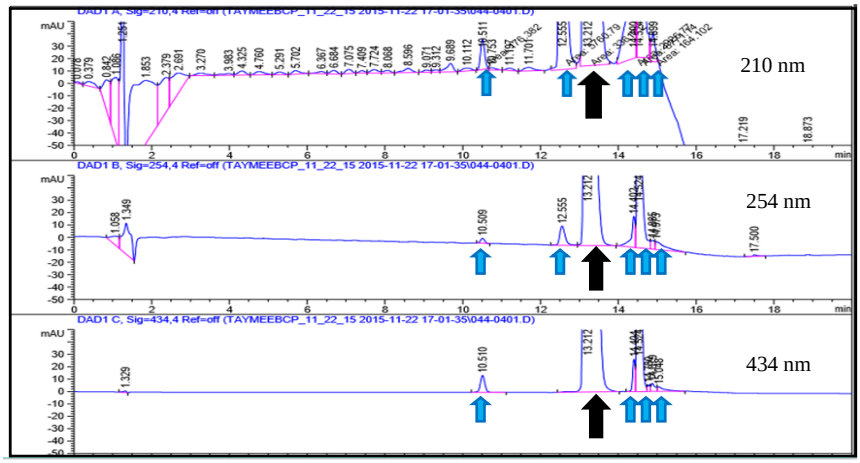
In Figure 10, the arrows indicate which peaks have a spectra associated with them, and are considered peaks of interest. ACN is a polar solvent and has an associated absorbance that is reflected on the chromatogram; when the instrument pumps solvent through the column, the detector registers the pulse of solvent, which is shown by the baseline waves seen between peaks of interest. At 13 minutes, the solvent profile increases to 100% acidified ACN to clear the column of contaminants. This quick increase causes the drastic baseline shift seen around this time in the chromatograms in Figure 10. The reason for the three separate wavelengths is to reveal the nature of the contaminants. At 210 nm, all portion of the mixture absorb, as well as the solvent. At 434 nm, sulfonephthalein indicators absorb, so all peaks at this wavelength consequently represent an indicator. This is the most important wavelength, because when in situ instruments such as SAMI's ™ are using indicators to collect data, they collect values at this wavelength; if there are contaminants that are indicator in nature, their absorbance will be included in the calculations and increase the margin of error.



A) HPLC-UV/Vis chromatogram of impure BCP solution at 790 ppm in 20% ACN: 0.10%TFA:80% H_2O to use as visual comparison of the chromatograms for Collections 1 and 2 from Figure 7. The method seen in Figure 9 was used with conditions listed in Table 2.



B) HPLC-UV/Vis chromatogram of a sample taken directly from Collection 1 seen in Figure 7. No further dilution was performed. The method seen in Figure 9 was used with conditions listed in Table 2.



C) HPLC-UV/Vis chromatogram of a sample taken directly from Collection 2 seen in Figure 7. No further dilution was performed. The method seen in Figure 9 was used with conditions listed in Table 2.

Note: ⇧ Arrows indicate spectral data ⬆ BCP ⬆ Contaminants of significance for % change in purity calculations

Figure 10: Comparison of HPLC-UV/Vis data for Collections 1 and 2 from Figure 7

Results and Discussion

Data Analysis

Following an effective purification and an apparent resolution of contaminants shown by the quality control data, data analysis can be performed to provide quantitative information to verify the purification. Peaks, such as those shown in Figure 10, have an associated area (mAU*s). The area is a function of absorbance and time, and using the fundamental basis of the Beer-Lambert law (Equation 2), a relative contaminant resolution calculation can be performed, seen in Table 3.

WAVELENGTH OF INTEREST	RATIO PURIFIED	RATIO UNPURIFIED	% CHANGE IN PURITY	
210 nm	9.74E-02	1.33E-01	26.9%	
254 nm	1.50E-02	7.89E-02	80.9%	
434 nm	3.71E-04	1.51E-02	97.5%	
% Yield	~30%			
% Contaminants in impure BCP	~1.5% @ 434 nm			
% Contaminants in purified BCP	~3.7E-04% @ 434 nm			
Equation 3:	$\%\ Change\ in\ Purity = 100 * \left(\frac{\sum \frac{x_{n\ pure}}{BCP_{pure}} - \sum \frac{x_{n\ impure}}{BCP_{impure}}}{\sum \frac{x_{n\ impure}}{BCP_{impure}}} \right)$ with x_n= area of contaminant n @ t_r n (mAU*s)			

Table 3: Data analysis of HPLC-UV/Vis data shown in Figure 10 using Equations 3, 4, and 5

To determine the contaminant resolution in the purified indicator, a calculation using the summation of the peaks of significance is shown in Table 3. Any peak with an associated spectra is considered a peak of significance. Peak spectra were used to verify contaminant peaks versus sulfonephthalein indicator peaks based on absorbance. Percent yield is calculated using Equation 4: *(Actual Yield/Theoretical Yield) x 100 = % Yield,* and the overall percent contaminants is estimated using Equation 5: $[1-(\Sigma x_{n\ contaminants}/\Sigma x_{n\ all\ peaks})] \times 100 = \%\ Contaminants.$

At each wavelength, there is a definite resolution of contaminants, but most notably at 434 nm where sulfonephthalein indicators absorb. This nearly complete resolution of the contaminants, specifically 97.5% as shown in Table 3, supports the conclusion that the proposed method created from this study effectively purifies BCP for seawater alkalinity titrations. The implications of this purification is that the purified BCP will create less interference with the

reduced amount of unknown contaminants. In addition, the nearly completely resolved contaminants at the sulfonephthalein wavelength of 434 nm will prevent additional indicators from affecting the calculations used to measure oceanic pH and alkalinity.

Summary/Conclusions

Based on the data analysis performed, and consistency of the results, the procedure produced in this study effectively purifies BCP at a level useful for seawater alkalinity titrations. Now that BCP is purified, the indicator needs to be fully characterized to reevaluate the molar absorptivity value ratios and pK_a using 2-aminopyridine as a pH buffer; 2-aminopyridine has been presented by Andrew G. Dickson as an acceptable pH buffer to be used for sea water media based on the total hydrogen ion concentration scale[1]. The buffer was chosen to use in future characterization work based on it having similar properties to BCP. Once characterization is finished in the near future, the more accurate constants will reduce the error in pH calculations, allowing for an improved understanding of the current state of ocean acidification.

Acknowledgement

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